Abstracts

Plenary Session

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The importance of newborn screening in defining the natural history of congenital cytomegalovirus infection

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Background: Congenital human cytomegalovirus (cCMV) infection represents the most common intrauterine viral infection in humans. The prevalence of cCMV ranges from 0.5–2 % in live births. cCMV represents a significant cause of long term neurological disease with approximately 5 % of infected infants developing cognitive and/or motor impairment and 11–15 % suffering hearing loss.

Methods: Early screening programs used virus isolation and immunological detection assays. Dried blood spots obtained for newborn metabolic screening have also been used in screening programs. More recently, we reported screening for cCMV using saliva and PCR based detection of CMV. Because the commitment of resources for the care of children with cCMV will depend on the prevalence and the outcome of infected infants, simplified screening programs represent an important advance. Estimates based on the identification of cCMV infected infants using clinical criteria are subject to enrollment biases. To determine the importance of such bias in natural history studies of cCMV, we reviewed the records of a large cohort of infants identified and followed at UAB from the 1970s through 2000. Infants enrolled in this clinic included screened newborns born at a University hospital and a second group referred from outside health care facilities secondary to clinical findings consistent with cCMV.

Discussion: In earlier reports as well as recent meta-analyses, it has been suggested that infants with clinically apparent cCMV (symptomatic infections) had a significant risk for neurological sequelae. Our findings revealed that the severity of symptoms and long-term neurological sequelae were significantly increased in the referred population as compared to the screened population.

Conclusion: Combining these populations lead to a bias in the results from these earlier studies and more importantly, lead investigators to conclude that the risk for long term neurological sequelae following cCMV could be assigned based on the presence of symptomatic infection. In contrast, our results indicate that cCMV infections in the newborn infant represent a spectrum of disease phenotypes and that risks of long-term neurological sequelae also varies with the nature of clinical abnormalities at birth.

Key words: newborn infant, human cytomegalovirus, congenital, neurological sequelae

Life course perspective of the origin of cardiovascular disease in women

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Background: Cardiovascular disease (CVD) is the leading cause of death in developed countries and slightly less in developing countries. The progression of risk factors from fetal life and childhood and CVD risk factors during pregnancy are now providing epidemiologists & clinicians opportunities for identification and prevention of early CVD in women soon after pregnancy. Large cohort studies have clearly shown that women who develop hypertension, dysglycemia during pregnancy or who deliver low birth weight or preterm infants are at significant risk of developing CVD earlier than women who have a normal pregnancy.

Objectives: For the past 20 years our research team has been doing routine uterine artery vascular resistance assessment during pregnancy to identify those at risk for poor fetal growth. In 2009 (Placenta) we published results on 523 subjects beginning at 19–21 wks, repeated at 29–31wks and at 34–36wks. 63 women delivered PT (32 spontaneous & 21 indicated.). We observed significantly elevated uterine artery (UtA) vascular resistance (VR) at each time point compared to those delivering at term. On the fetal side of the placenta at 19–21 wks there was no difference in fetal umbilical artery (UmA) VR between those delivering PT vs. term; however, at 28–31wks and at 34–36wks those infants delivering PT had significantly elevated UmAVR. We now interpret this finding as the first observation of "fetal programming" where the fetuses destined to deliver PT recognized the reduced UtA perfusion and developed increase UmAVR that persisted until delivery.

Conclusions: Based upon the literature prior to 2001 Thorp JM (Curr Probl Obstet Gynecol Fertil) proposed a unifying hypothesis of placental vascular compromise for PTB. Our research team considers UtAVR in women destined to deliver PT is evidence of early maternal cardiovascular disease which is consistent with epidemiological evidence for why women with PTD, Preeclampsia/Hypertension, Obesity & Dysglycemia are at risk for early cardiovascular disease.

Posttranslational titin isoform modifications in perinatal diastolic dysfunction

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In newborns, the background of diastolic dysfunction (leading potentially to heart failure with preserved ejection fraction, HFPEF) is unknown. Earlier, we have pointed out the significance of cardiomyocyte passive force increase (Fpassive=tension determined in Ca^{2+}-free solution) in the pathogenesis of adult HFPEF. One of the most important determinants of Fpassive is the giant sarcomeric protein, titin. We hypothesize, that physiological and pathological adaptation processes to the extrauterine life involves posttranslational modifications (phosphorylation and oxidation) of differentially expressed titin isoforms, and that these changes together coordinate Fpassive of newborns. Therefore, we will follow the titin isoform dependence of Fpassive in permeabilized cardiomyocytes during the perinatal period in control and in transgenic mice overexpressing the phosphodiesterase 5 isoform. In addition, in model experiments on cardiomyocytes of mice, healthy rats at different stages of postnatal development and of adult humans we will test how *in vitro* protein kinase A (PKA), or protein kinase G (PKG) exposures, alone or in combination with oxidative insults (i.e. SH-group oxidation, carbonylation) affect Fpassive. In a parallel clinical study, diastolic ventricular function and oxidative plasma/urine markers will be followed to reveal their hypothetical relationship during the postnatal period in term and preterm human newborns. Altogether, our investigations will clarify the pathomechanism of perinatal diastolic dysfunction due to inappropriate cardiovascular adaptation to the extrauterine life and promote the development of new approaches for its pharmacological management.

Risk factors for ischemic heart disease in the Croatia, Czech Republic, Hungary and Romania.

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BACKGROUND: Despite of the strong prognostic value of all traditional cardiovascular risk factors for ischemic heart disease (IHD), differences exist in the incidence of clinical events between patients at apparently similar risk. One of the reasons could be different genetic background of particular person. Interpretation of genetic factors associated with IHD is complicated by different prevalence of risk factors in controls. In our previous studies we observed association between connexin37 gene polymorphism and IHD, limited only to non-smoking women. In this presentation we focused on complex and gender oriented statistical analyses of differences in main cardiovascular risk factors between patients with IHD from four European countries.

METHODS: Population consisted of 1,735 men and 661 women with IHD. Gender, smoking status, presence of hypertension, diabetes mellitus, and polymorphism of connexin 37 gene were analyzed. The control population consisted of 1,191 men and 1,368 women from the post-MONIKA study. For statistical analyses chi2 square test was used.

RESULTS: In the whole group, all risk factors were more prevalent in patients than in controls: smoking-67.7 vs. 46.7 %, $p < 0.0001$; diabetes mellitus-20.6 vs. 13.9 %, $p< 0.0001$; hypertension-61.8 vs. 37.7 %, $p < 0.0001$. No difference was found for connexin37 genotypes between patients and controls (CC/CT/TT): 10.4/43.0/46.5 vs. 9.6/44.0/46.4 5 %, $p = 0.6102$. Regarding diabetes mellitus, difference between patients and controls was found in women: 25.7 vs. 11.7 %, $p < 0.0001$; but not for men 18.8 vs. 16.5 %, $p = 0.1241$. No differences were found between men and women in other risk factors under study.

CONCLUSION: In the complex statistical evaluation, the only gender-related difference in association between traditional cardiovascular factors and IHD was found in the case of diabetes mellitus, which was associated with IHD only in women. No association was found between polymorphism of the gene for connexin37 and ischemic heart disease using complex statistical analyses. According to our previous results, also stratification of patients according to prevalence of cardiovascular risk factors could reveal gene traits associated with ischemic heart disease in particular populations.

Key words: cardiovascular risk factors- ischemic heart disease – gender differences - connexin 37 gene polymorphism

Vascular responses to various physiological conditions

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The endothelium plays an important role in maintaining vascular homeostasis in various physiological conditions. Several endothelium-derived factors were shown to mediate vascular responses, among which the metabolites of arachidonic acid (AA) have very important role. AA could be metabolized via three pathways:
via cyclooxygenases (COX) to prostaglandins and thromboxanes,
via lypooxygenase to leukotrienes and
via P450 (CYP) pathway to epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE). Each of them has particular effect on vascular tone and subsequently tissue perfusion. Our recent studies demonstrated the role for CYP metabolite in vascular responses to vasodilator stimuli, such as acetylcholine and reduced pO2 in hyperbaric oxygenation conditions.

1001 Complement tales: carbon nanotubes, poly(ethylene glycol)s and the just so forty complement proteins

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'*Verily the works and words of our ancestors have become signs and examples to people of our modern age so that they may view what happened to other folk and take heed; so that they may peruse the annals of ancient peoples and read about everything they have experienced and thereby be guided and restrained.*' [Prologue: Arabian Nights: A Selection; Translated by Sir Richard F. Burton, Penguin]

Not so long ago and still carbon nanotubes (CNTs) are in contention for site-specific drug and nucleic acid delivery, photodynamic therapy, and photoacoustic molecular imaging, particularly in experimental oncology [1]. Pristine carbon nanotubes are insoluble in nearly all aqueous solvents and biological fluids. Accordingly, there have been many measures in terms of surface treatment and functionalization strategies to render nanotubes readily dispersible [1–3]. PEGylation is a widely used surface modification approach, which not only renders pristine CNTs dispersible in aqueous solvents, but also improves their circulation profiles in the vasculature [1, 3–6]. PEGylation, however, may trigger the complement system, which is an integral part of innate immunity, where its inadvertent activation can induce clinically significant anaphylaxis [2, 3, 7–9]. In this presentation, I shall demonstrate that covalent functionalization of CNTs with poly(ethylene glycol)s, regardless of PEG molecular mass and surface density, fails to protect against complement activation [6]. On the other hand, surface adsorbed methoxypoly(ethylene glycol)-based amphiphiles, which also confer solubility and prolonged circulation profile to CNTs, can modulate activation of human complement system differently, depending on the amphiphile structure. While immobilized linear poly(ethylene glycol) amphiphiles fully trigger complement through both L-ficolin and mannose-binding lectin sensing, high molecular weight amphiphiles with branched poly(ethylene glycol) architecture neither initiate anaphylatoxin generation nor induce triggering of the effector arm of the complement system, despite activating calcium-sensitive pathways of the complement system [5]. Finally, increased PEG loading, through adsorption of methoxypoly(ethylene glycol) amphiphiles on covalently PEG-functionalized CNTs, generate fewer complement activation products; however, complement activation is not completely eliminated [6]. These observations address two critical issues: (a) the difficulty in making CNTs more compatible with innate immunity through PEG functionalization and adsorption, and (b) offering a critical step towards nanomaterial surface modification with branched methoxyPEG co-polymers with appropriately distanced PEG chains for improved protein exclusion that can significantly improve innate immunocompatibility. However, with the latter case problems still

persist; the high molecular weight of the copolymer limits its eventual excretion through kidneys, where the cutoff for glomerular filtration is 60 kDa.

'*And yet, oh king, this tale is no more wondrous than the remarkable story of the blood-traveller*.' To be continued.

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References

1. Andersen, A. J., Wibroe, P. P. and Moghimi, S. M. (2012) Perspectives on carbon nanotube-mediated adverse immune effects. Adv. Drug Deliv. Rev. 64: 1700–1705.
2. Moghimi, S. M. and Hunter, A. C. (2010) Complement monitoring of carbon nanotubes. Nature Nanotechnol. 5: 382–382.
3. Moghimi, S. M., Andersen, A. J., Hashemi, S. H., Lettiero, B., Ahmadvand, D., Hunter, A. C., Andresen, T. L., Hamad, I. and Szebeni, J. (2010) Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: the challenges ahead. J. Control. Rel. 146: 175–181.
4. Moghimi, S. M., T. L. Andresen and Hunter, A. C. (2012) Factors controlling nanoparticle pharmacokinetics: an integrated analysis and perspective. Annu. Rev. Pharmacol. Toxicol. 52: 481–503.
5. Andersen, A. J., Robinson, J. T., Dai, H., Hunter, A. C., Andresen, T. L. and Moghimi, S. M. (2013) Single-walled carbon nanotubes surface control of complement sensing and activation. ACS Nano 7: 1108–1119.
6. Andersen, A. J., Windschiegl, B., Ilbasmis-Tamer, S., Degim, I. T., Hunter, A. C., Andresen, T. L. and Moghimi, S. M. (2013) Complement activation by PEG-functionalized MWCNTs is independent of PEG molecular mass and surface density. Nanomedicine: Nanotechnol. Biol. Med. (in press).
7. Hamad, I., Al- Hanbali, O., Hunter, A. C., Rutt, K. J., Andresen, T. L. and Moghimi, S. M. (2010) Distinct polymer architecture mediates switching of complement activation pathways at nanosphere-serum interface: implications for stealth nanoparticles engineering. ACS Nano 4: 6629–6638.
8. Moghimi, S. M., Wibroe, P. P., Helvig, S., Farhangrazi, Z. S. and Hunter, A. C. (2012) Genomic perspectives in inter-individual adverse responses following nanomedicine administration: the way forward. Adv. Drug Deliv. Rev. 64: 1385–1393.
9. Moghimi, S. M. and Farhangrazi, Z. S. (2013) Nanomedicine and the complement paradigm. Nanomedicine: Nanotechnol. Biol. Med. (in press).